IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Patent Application of Meir Shinitzky
Serial No. 09/937,386 Group Art Unit: 1626
Filed: March 24, 2000 Examiner: Ebenezer Sackey

Declaration
Under Rule 132

Commissioner of Patents and Trademarks
Washington, D.C. 20231

I, Meir Shinitzky, an Israeli citizen residing at Derech Haganim Street 20, Kfar Shmaryahu, Israel 46910 hereby declare:

1. I am a tenured professor of Biological Chemistry at the Weizmann Institute of Science, Rehovot Israel since 1978. My areas of expertise include synthesizing and preparing new compounds and their use for promoting cellular reactions.

2. A brief list of recent (5 years) relevant publications is enclosed herewith as Annex "A" at the end of the present Declaration.

3. I am the inventor of the above-mentioned U.S.A. Patent Application titled "*Cyclic Glycerophosphates and Analogues Thereof*" (hereinafter "*the application*"). The application describes pharmaceutical compositions containing as the active component a cyclic glycerophosphate and use of such pharmaceutical compositions for promoting cell differentiation and treating various malignancies.

4. Since filing the application we have carried out additional experiments supporting the subject matter of the application. In particular the additional experiment which was done showing a positive effect of inhibiting human prostate cancer by a pharmaceutical composition containing cyclic glycerophosphate supports claimed subject matter of the application. The results on inhibition of human prostate cancer given in **Annex "B"** taken together with the experimental data given in the application support the approach of the application which is hormonal dependent cancer therapy.

5. Normal mature breast cells possess a high level of receptors for the steroidal hormones estrogen and progesterone. For the sake of clarity it may be added that during pregnancy, the level of the hormones may be 10 fold thereby resulting in enhanced activity of the breast cells, resulting in the production of milk. Cancer cells, are far from being mature, are not differentiated and thus contain very low levels of receptors for these steroidal hormones. The level of differentiation of

breast cancer cells may easily be determined pathologically in order to determine their maturation. As the differentiation level of breast cancer cells increase they become closer and closer to be "normal" cells (regarding their fenotip). As the differentiation level increases the rate of proliferation is normal. It is desirous to obtain cancer cells which are normal cells (differentiation and proliferation) since at such a stage these cancerous cells may be treated by estrogen blockers or by antagonists to the estrogen e.g. tamoxifen.

6. Put in other words, non mature breast cancer cells have a low level of hormone receptors and thus are difficult to treat since they are abnormal cells. Increasing the differentiation of breast cancerous cells leads to the formation of "normal-like" breast cancer cells and this has two positive effects. One is that "normal-like" breast cancer cells have normal levels of hormone receptors, which can then be treated with estrogen blockers or antagonists which cause death of the cells. Thus such "normal-like" cancer cell may be treated more efficiently. The other benefit is that developed differentiation leads to longer proliferation time, thus slowing the entire growth rate.

7. Treatment of human prostate cancer is similar to that of breast cancer since as in the breast, prostate cells contain high levels of receptors for a steroidal hormone. The hormone in the case of prostate is a short peptide identified as LH. Analogically, enhancing the differentiation of prostate cancer cell would bring them to be "normal-like" prostate cells that can then be treated with blockers or antagonists of the hormone peptide. Hormonal treatment of prostate cancer is known by using antiandrogenic agents. Thus the additional results presented in Annex "B" with regards to human prostate cancer demonstrate the approach of the present invention with regards to hormonal dependent cancer therapy.

8. I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: Dec. 11 03

Prof. Meir Shinitzky

Annex A

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153. Deckmann, M., Mamillapalli, R., Schechtman, L. and Shinitzky, M. A conformational epitope which detects autoantibodies from schizophrenic patients. Clin. Chim. Acta. 322:91-98 (2002).

154. Shinitzky, M., Nudelman, F., Barda, Y., Haimovitz, R. Chen, E. and Deamer, D.W. Unexpected differences between D- and L- tyrosine lead to chiral enhancement in racemic mixtures. Origins of Life and Evolution of the Biosphere 32:285-297 (2002).

155. Adan, Y., Goldman, Y., Haimovitz, R., Mammon, K. Tamarin-Povolotsky, V., Pressman, E. and Shinitzky, M. Elevation of steroid hormone receptors in MCF-7 breast cancer cells by 1,3 cyclic propanediol phosphate. Cancer Letters. In press.

Annex B

In Vivo Experiment of Human Prpstate Cancer Cells By 1, 3 Cyclic Propanediol Phosphate.

Materials and methods

Cells

The human prostate cancer CL-1 cell line was a gift of Prof. Zelig Eshhar, of our Institute. The cells were grown as monolayers in Rosewell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% FCS, 2 mM glutamine, and antibiotics. The CL-1 is a poorly differentiated cell line derived from a sub-clone of LNCaP (metastatic lymph node). This cell line was grown at 37 °C in the presence of 5% CO_2. The absence of *Mycoplasma* contamination was monitored by a polymerase chain reaction (PCR) assay carried out once every 3 months.

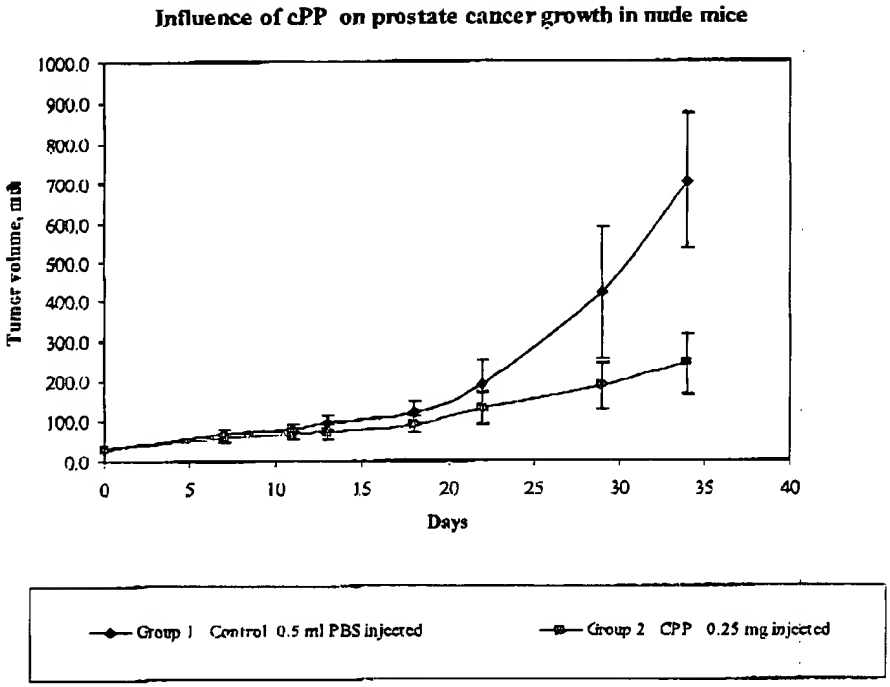
cPP

This compound was synthesized, purified and was dissolved in Hanks' balanced salt solution or cell culture medium and sterilized by filtration.

In Vivo experiment

Male CD-1 nude mice, 6 weeks age, were obtained from the animal breeding center of our Institute. Animals were maintained and treated according to 'Principles of laboratory animal care', under the supervision of the Council for Experiments on Animals of our Institute. Mice were anesthetized and implanted subcutaneously with 10^6 CL-1 cells in the dorsal space. Twelve days later, when the tumors became palpable, mice were randomly allocated into two groups, each with ten mice. The control group was injected with PBS and the treatment group was injected with 0.25 mg 1,3cPP/mouse in PBS i.p. in the lower left abdomen once daily, 5 times weekly, for three weeks. Tumor diameter was serially measured with a caliper assuming a hemiellipsoid shape, where VOLUME = (4π /3) × (length/2) × (width/2) × (thickness/2). There were no changes in appearance, body weight normal behavior upon injection of 1,3cPP over the course of the experiment.

Results


Influence of cPP on prostate cancer growth in nude mice
Tumor volume, mm3
1000.0
900.0
800.0
700.0
600.0
500.0
400.0
300.0
200.0
100.0
0.0
0
5
10
15
20
25
30
35
40
Days
Group 1 Control 0.5 ml PBS injected
Group 2 CPP 0.25 mg injected